

## CLAIMS

1. A *PRC1* gene derived from *Hansenula polymorpha* DL1 (ATCC 26012), having the base sequence listed in Sequence 1, which codes for carboxypeptidase Y.
2. A vector pHYL, which is constructed by inserting a *Hansenula polymorpha* *LEU2* gene into a plasmid pHDY2 (KCTC 0732BP) carrying the *PRC1* gene of Sequence 1 in such a way that the *PRC1* gene is disrupted.
3. A vector pHYUZ, which is constructed by inserting a *Hansenula polymorpha* *URA3* gene pop-out cassette into a plasmid pHDY2 carrying the *PRC1* gene of Sequence 1 in such a way that the *PRC1* gene is disrupted.
4. A carboxypeptidase Y mutant strain, into which a *Hansenula polymorpha* UR2 strain is transformed with the vector pHYL of claim 2.
5. A carboxypeptidase Y mutant strain, *Hansenula polymorpha* DL1/ $\Delta$ cpy(KCTC 0735 BP), into which a *Hansenula polymorpha* DL1 strain is transformed with the vector pHYUZ of claim 3.
6. A process for producing a recombinant protein, wherein a gene coding for an exogenous protein is introduced into the carboxypeptidase Y mutant strain of claim 4 and the cell is cultured in a culture medium.
7. A process for producing a recombinant protein, wherein a gene coding for an exogenous protein is introduced into the carboxypeptidase Y mutant strain of claim 5 and the cell is cultured in a culture medium.
8. A recombinant protein, which is produced by the process of claim 6 or 7.
9. A *PEP4* gene derived from *Hansenula polymorpha* DL1 (ATCC 26012),

0000192-11401  
FOH11-26E000

having the base sequence listed in Sequence 2, which codes for protease A.

10. A vector pHPL, which is constructed by inserting a *Hansenula polymorpha* *LEU2* gene into a plasmid pHDP4 (KCTC 0733BP) carrying the *PEP4* gene of Sequence 2 in such a way that the *PEP4* gene is disrupted.

5 11. A vector pHPUZ, which is constructed by inserting a *Hansenula polymorpha* *URA3* gene pop-out cassette into a plasmid pHDP4 carrying the *PEP4* gene of Sequence 2 in such a way that the *PEP4* gene is disrupted.

12. A protease A mutant strain, into which a *Hansenula polymorpha* UR2 strain is transformed with the vector pHPL of claim 10.

10 13. A protease A mutant strain, *Hansenula polymorpha* DL1/ $\Delta$ pep4 (KCTC 0734 BP), into which a *Hansenula polymorpha* DL1 strain is transformed with the vector pHPUZ of claim 11.

14. A process for producing a recombinant protein, wherein a gene coding for an exogenous protein is introduced into the protease A mutant strain of claim 12 and the  
15 cell is cultured in a culture medium.

15. A process for producing a recombinant protein, wherein a gene coding for an exogenous protein is introduced into the protease A mutant strain of claim 13 and the cell is cultured in a culture medium.

16. A recombinant protein, which is produced by the process of claim 14 or 16.

20 17. A *KEX1* gene derived from *Hansenula polymorpha* DL1 (ATCC 26012), having the base sequence listed in Sequence 3, which codes for carboxypeptidase  $\alpha$ .

18. A vector pKUZ, which is constructed by inserting a *Hansenula polymorpha*

09993192-111401

*URA3* gene pop-out cassette into a plasmid pKH3.9 carrying the *KEX1* gene of Sequence 3 in such a way that the *KEX1* gene is disrupted.

19. A carboxypeptidase  $\alpha$  mutant strain, *Hansenula polymorpha* DL1/ $\Delta$ kex1(KCTC 0736 BP), into which a *Hansenula polymorpha* DL1 strain is  
5 transformed with the vector pKUZ of claim 18.

20. A process for producing a recombinant protein, wherein a gene coding for an exogenous protein is introduced into the carboxypeptidase  $\alpha$  mutant strain of claim 19 and the cell is cultured in a culture medium.

21. A recombinant protein, which is produced by the process of claim 20.

10 22. A carboxypeptidase Y/protease A mutant strain, into which a *Hansenula polymorpha* DL1 strain is transformed with the vector pHYUZ of claim 3 and the vector pHPUZ of claim 11.

23. A process for producing a recombinant protein, wherein a gene coding for an exogenous protein is introduced into the carboxypeptidase Y/protease A mutant strain of  
15 claim 22 and the cell is cultured in a culture medium.

24. A recombinant protein, which is produced by the process of claim 23.

25. A carboxypeptidase Y/carboxypeptidase  $\alpha$  mutant strain, into which a *Hansenula polymorpha* DL1 strain is transformed with the vector pHYUZ of claim 3 and the vector pKUZ of claim 20.

20 26. A process for producing a recombinant protein, wherein a gene coding for an exogenous protein is introduced into the carboxypeptidase Y/carboxypeptidase  $\alpha$  mutant strain of claim 25 and the cell is cultured in a culture medium.

09993192.111401

28. A recombinant protein in accordance with claim 27, wherein the recombinant protein is human epidermal growth factor (hEGF).

10 31. A recombinant protein, which is produced by the process of claim 30.

33. A process for producing a recombinant protein, wherein a gene coding for an  
15 exogenous protein is introduced into the carboxypeptidase Y/protease  
A/carboxypeptidase  $\alpha$  mutant strain of claim 32 and the cell is cultured in a culture  
medium.

34. A recombinant protein, which is produced by the process of claim 33.

35. A vector pMLT-delta (KCTC 0727BP), which carries as a selective marker  
20 for *Hansenula polymorpha* a *Saccharomyces cerevisiae* *LEU2* inserted between a portion  
of a *MOX* promoter and a DNA fragment comprising a portion of a *TRP3* gene  
(*mox(p)::S.cerevisiae LEU::trp3*).

36. A *Hansenula polymorpha*  $\Delta$ mox mutant strain, which is prepared by introducing the vector pMLT-delta of claim 35 into *Hansenula polymorpha* in such a way that the *MOX-TRP3* gene of *Hansenula polymorpha* is disrupted.

37. A *Hansenula polymorpha*  $\Delta$ mox mutant strain in accordance with claim 36,  
5 wherein the *Hansenula polymorpha* is a *Hansenula polymorpha* DL1 strain.

38. A *Hansenula polymorpha*  $\Delta$ mox mutant strain DLT2 (KCTC 0728BP), which is prepared by introducing the vector pMLT-delta of claim 35 into *Hansenula polymorpha* DL1-L in such a way that the *MOX-TRP3* gene of *Hansenula polymorpha* is disrupted.

10 39. A process for producing a recombinant protein, wherein the *Hansenula polymorpha*  $\Delta$ mox mutant strain of any of claims 36 to 38 is transformed with an expression cassette and cultured in a methanol medium, serving as a recombinant protein expression host, said expression cassette comprising a promoter whose expression is induced by methanol.

15 40. A process in accordance with claim 39, wherein the *Hansenula polymorpha*  $\Delta$ mox mutant strain is first cultured to a high concentration in a medium containing glycerol as a carbon source and then, cultured in a medium containing methanol as a carbon source.

41. A recombinant protein, which is produced by the process of claim 39 or 40.

20 42. A process for popping-out an expression cassette integrated into the *MOX* gene site of the  $\Delta$ mox strain DLT2.

43. A process for developing novel mutant strains derived from a recombinant DLT2 strain, which is prepared in accordance with claim 38, into a host for general use in producing various recombinant proteins using the pop-out technique of claim 42.

09993192.111401